THREE DIMENSIONAL COORDINATES OF HPTPbeta

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the priority under Title 35 U.S. Code 119(e) from Provisional Application Serial No. 60/413,547 filed September 25, 2002, which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to three-dimensional structures of the catalytic domain of HPTPbeta, and structures derived therefrom.

BACKGROUND OF THE INVENTION

HPTPbeta (Kruegar et al., EMBO J., 9, (1990)) has been suggested *inter alia* for modulating the activity of angiopoietin receptor-type tyrosine kinase Tie-2. See PCT Patent Application WO 00/65088. Inventors have shown in the present and concurrently filed patent applications that modulation of HPTPbeta modulates activities of both Tie-2 and VEGFR2. Therefore, HPTPbeta could be a target for the treatment of angiogenesis mediated disorders. However, the crystal structure of HPTPbeta has not been described. High-resolution 3D experimental models are needed to obtain insight into the mechanisms of HPTPbeta activation, the source of interactions between specific ligands and HPTPbeta, and to design better agonists and antagonists of HPTPbeta. Thus, there is a need for crystal structure of HPTPbeta.

SUMMARY OF THE INVENTION

The present invention attempts to address this need by providing a 3D structure of the catalytic domain of human HPTPbeta, and suitable means to design and identify potent and selective agonists or antagonist of the HPTPbeta for the treatment of angiogenesis mediated disorders.

In one aspect the invention provides for crystalline forms of the HPTPbeta catalytic domain having unit cell dimensions of a=62±1 Å, b=72±1 Å, and c=70±1 Å, α=90°, β=93±3°, γ=90° in the space group $P2_1$ (monoclinic form) and unit cell dimensions of a=39±1 Å, b=71±1 Å, and c=120±2 Å, α=90°, β=90°, γ=90° in the space group $P2_12_12_1$ (orthorhombic form).

In another aspect the invention provides for a method of identifying a compound useful for the treatment of an angiogenesis mediated disorder, comprising the steps of using a three-dimensional (3D) structure of HPTPbeta as defined by the atomic coordinates of Figures 7-304, or combination thereof; and employing said 3D structure to design, modify, or select a compound that binds HPTPbeta *in silico*.

A method of identifying a compound useful for the treatment of an angiogenesis mediated disorder, comprising the steps of: providing a crystal of the complex between HPTPbeta and compound, or alternately exposing a crystal of HPTPbeta with a compound in aqueous media; exposing the complex to X-rays to generate a diffraction pattern; capturing the pattern to a recording device to generate diffraction data; processing the data to solve the complex structure; determining the location of compound within complex structure; wherein the compound binding to the binding site of HPTPbeta, wherein the binding site is selected from the group consisting of P0, P+1, P-1, or mixtures thereof, indicates the compound is useful for the treatment of the HPTPbeta mediated disorder.

A method of identifying a compound useful for the treatment of an angiogenesis mediated disorder, comprising the steps of: selecting the compounds based on computer-aided drug design (CADD) using the coordinates from Figures 7-304; further analyzing if the compound binds HPTPbeta or modulates HPTPbeta activity in an *in vitro*, *in vivo*, or *ex vivo* assay; and identifying those compounds that bind HPTPbeta or modulate HPTPbeta activity as compounds useful for the treatment of an angiogenesis mediated disorder.

These and other objects, features, and advantages will become apparent to those of ordinary skill in the art from a reading of the following detailed description and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS AND TABLES

Figure 1 shows a ribbon representation of the carbon-alpha trace of the HPTPbeta catalytic domain.

Figure 2 shows the change that occurs in the WPD loop of the HPTPbeta catalytic domain upon ligand binding (ligand-free structure is shown as darker trace).

Figure 3 shows a superposition between Compound 1 ((S)-[1-Methylcarbamoyl-2-(4-sulfoamino-phenyl)-ethyl]-carbamic acid benzyl ester) (darker) and Compound 2 ({2-(4-Hydroxy-

phenyl)-1-[1-methylcarbamoyl-2-(4-sulfoamino-phenyl)-ethylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester) (lighter) structures bound to HPTPbeta catalytic domain.

Figure 4 schematically represents interactions between Compound 1 and the HPTPbeta catalytic domain: (a) hydrogen bonding and (b) Van der Waals interactions. The ligand is shown in magenta, the main body of the protein is colored blue, and the WPD loop residues are colored red.

Figure 5 shows an overlay of the phosphotyrosine (darker) bound to PTP-1B trap mutant and Compound 1 (lighter) bound to the HPTPbeta catalytic domain.

Figure 6 shows Tyr212 conformation in enzyme complex with Compound 1 (lighter) and Compound 2 (darker).

Figure 7-102 show the atomic structure coordinates for HPTPbeta as derived from a monoclinic crystal of ligand-free HPTPbeta catalytic domain polypeptide.

Figure 103-201 show the atomic structure coordinates for HPTPbeta and the inhibitor molecule as derived from a monoclinic crystal of HPTPbeta bound to the inhibitor Compound 1.

Figure 202-252 show the atomic structure coordinates for HPTPbeta as derived from an orthorhombic crystal of ligand-free HPTPbeta catalytic domain polypeptide.

Figure 253-304 show the atomic structure coordinates for HPTPbeta and the inhibitor molecule as derived from an orthorhombic crystal of HPTPbeta bound to the inhibitor Compound 2.

The data shown in Figures 7-304 are expressed based on the Protein Data Bank (PDB) format: The PDB format is a format containing coordinates (X, Y, Z,), etc. of individual atoms, and is the standard formats in expressing coordinates of biopolymers. In Figures 7-304, the "ATOM" appearing in the utmost left column (1st column) denotes each atom of the atomic coordinates. The numbers (1, 2, 3, . . . etc.) appearing in the next column (2nd column) are serial numbers of individual atoms. Subsequently, in the left to right direction in these Figures, there are denoted the type of each atom and its position in the amino acid to which it belongs (e.g., "CB", "CG", "SD") (in the 3rd column); the amino acid residue to which each atom belongs (three-letter abbreviations for amino acids, e.g. "MET", "ASN") (in the 4th column); the sequence number of the residue counted from the N-terminal (in the 5th column); X-coordinate (in angstrom (Å) unit) (in the 6th column); Y-coordinate

(in angstrom (Å) unit) (in the 7th column); Z-coordinate (in angstrom (Å) unit) (in the 8th column).

SEQUENCE LISTING DESCRIPTION

Each of the nucleotide or amino acid sequences in the sequence listing is shown in Table A.

Table A

Sequence Description	**SEQ ID NO: Nucleotide, Amino Acid**	**Species**	**Genbank (GB) or Derwent (D) Accession No. for Nucleotide Sequence**	**Related Genbank (GB) or Derwent (D) Accession Nos.**
HPTPbeta (HPTP-beta, PTPRB, PTPbeta, PTPB, R-PTP-beta)	1 (nucleotide)	*Homo Sapiens*	X54131	NM_002837
HPTPbeta (HPTP-beta, PTPRB, PTPbeta, PTPB, R-PTP-beta)	2 (amino acid)	*Homo Sapiens*	X54131	NM_002837
HPTPbeta intracellular domain (ICD)	3 (nucleotide)	*Homo Sapiens*		NM_002837
HPTPbeta intracellular domain (ICD)	4 (amino acid)	*Homo Sapiens*		NM_002837
HPTPbeta truncated ICD	5 (nucleotide)	*Homo Sapiens*		NM_002837
HPTPbeta truncated ICD	6 (amino acid)	*Homo Sapiens*		NM_002837

HPTPbeta crystallized ICD	7 (amino acid)	*Homo Sapiens*		NM_002837
Intracellular domain forward primer	8			
Intracellular domain reverse primer	9			
Crystal domain forward primer	10			
Crystal domain reverse primer	11			
Crystal, His-tag forward primer	12			
Crystal, His-tag reverse primer	13			
Crystal TEV-site, forward primer	14			
Crystal TEV-site, reverse primer	15			

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to identifying or obtaining compounds useful for modulating HPTPbeta activity. Crystal structure information presented herein is useful in designing compounds and modeling them or their potential interaction with binding site(s) of HPTPbeta. Actual compounds may be identified from following design and model work performed *in silico*. A

compound identified using the present invention may be effective for the treatment of an angiogenesis mediated disorder. These and other aspects and embodiments of the present invention are discussed below.

One aspect of the invention provides for the crystalline form of HPTPbeta. Four crystal structures of HPTPbeta are presented. Figures 7-102 show coordinates of HPTPbeta in the monoclinic crystal form having unit cell dimensions of a=61.89 Å, b=71.53 Å, and c=70.35 Å, α=90°, β=93.25°, γ=90° in the space group $P2_1$.

Figures 103-201 show coordinates of HPTPbeta in complex with Compound 1 in the monoclinic crystal form having unit cell dimensions of a=62.19 Å, b=71.80 Å, and c=70.45 Å, α=90°, β=93.56°, γ=90° in the space group $P2_1$.

Figures 202-252 show coordinates of HPTPbeta in the orthorhombic crystal form having unit cell dimensions of a=39.25 Å, b=71.13 Å, and c=119.91 Å, α=90°, β=90°, γ=90° in the space group $P2_12_12_1$.

Figures 253-304 show coordinates of HPTPbeta in complex with Compound 2 in the orthorhombic crystal form having unit cell dimensions of a=38.85 Å, b=69.61 Å, and c=117.78 Å, α=90°, β=90°, γ=90° in the space group $P2_12_12_1$. The structural data according to Figures 7-102; 103-201; 202-252; and 253-304 are at approximately 1.9, 1.8, 1.75, and 1.65 Angstrom (Å) resolution, respectively.

The coordinates of Figures 7-304 provide a measure of atomic location in Angstroms, to a first decimal place. The coordinates are a relative set of positions that define a shape in three dimensions. It is possible that an entirely different set of coordinates having a different origin and/or axes could define a similar or identical shape. Furthermore, varying the relative atomic positions of the atoms of the structure so that the root mean square deviation of the conserved residue backbone atoms (i.e. the nitrogen-carbon-carbon backbone atoms of the protein amino acid residues) is less than 1.5 Å, preferably less than 1.0 Å and more preferably less than 0.5 Å, when superimposed on the coordinates provided in Figures 7-304 for the conserved residue backbone atoms, will generally result in a structures which are substantially the same as the structures of Figures 7-304 in terms of both its structural characteristics and potency for structure-based drug design of HPTPbeta modulators. Likewise changing the number and/or positions of the water molecules of Figures 7-304 will not generally affect the potency of the structure for structure-based drug design of HPTPbeta modulators. Thus for the purposes described herein as being aspects of the present invention, it is

within the scope of the invention if: the coordinates of Figures 7-304 are transposed to a different origin and/or axes; the relative atomic positions of the atoms of the structure are varied so that the root mean square deviation of conserved residue backbone atoms is less than 1.5 Å (preferably less than 1.0 Å and more preferably less than 0.5 Å) when superimposed on the coordinates provided in Figures 7-304, respectively, for the conserved residue backbone atoms; and/or the number and/or positions of water molecules is varied. Reference herein to the coordinates of Figures 7-304 thus includes the coordinates in which one or more individual values of the Figures are varied in this way.

Also, modifications in the HPTPbeta crystal structure due to e.g. mutations, additions, substitutions, and/or deletions of amino acid residues could account for variations in the HPTPbeta atomic coordinates. However, atomic coordinate data of HPTPbeta modified so that a ligand that bound to one or more binding sites of the HPTPbeta binding pocket would also be expected to bind to the corresponding binding sites of the modified HPTPbeta, and therefore are, for the purposes described herein as being aspects of the present invention, also within the scope of the invention. References herein to the coordinates of Figures 7-304 thus include the coordinates modified in this way. Preferably, the modified coordinate data define at least one HPTPbeta binding site.

Another aspect of the invention provides for the HPTPbeta binding pocket, wherein the binding pocket comprises at least the P(0), P(1) and P(-1) binding sites. The nomenclature of the binding sites is based on binding of phosphorylated peptides to PTPases, for example: P(0) is the active site of the enzyme, which accommodates the phosphotyrosine residue of the phosphopeptide; P(+1) is the site which accommodates the amino acid of the phosphopeptide that is adjacent to the phosphotyrosine in the direction of the carboxy terminus of the phosphopeptide; P(-1) accommodates the amino acid of the phosphopeptide that is adjacent to the phosphotyrosine in the direction of the amino terminus of the phosphopeptide. In HPTPbeta, P(0) is characterized by at least amino acid residues 152, 74-77, 209-214, 244-253, 288-290, and 293 of SEQ ID NO: 7; P(+1) is characterized by at least amino acid residues 76-80, 48-66, 284-292, and 212-214 of SEQ ID NO: 7; P-1 is characterized by at least amino acid residues 69-76, 119-123, and 149-154 of SEQ ID NO: 7.

In Silico Drug Design

For the first time, the present invention permits the use of virtual design techniques (i.e., computer modeling or "*in silico*") to design, select, and synthesize compounds capable of

inhibiting/stimulating or binding HPTPbeta. In turn, these drug candidates may be effective in the treatment of an angiogenesis mediated disorder.

The term "angiogenesis mediated disorder" is defined as a disorder that involves a modulation in angiogenic activity resulting in the biological manifestation of a disease, disorder, and/or condition; in the biological cascade leading to the disorder; or as a symptom of the disorder. The Applicants have shown that the process of angiogenesis is modulated by HPTPbeta. This "involvement" of HPTPbeta in an angiogenesis mediated disorder includes, but is not limited to, the following: (1) The modulation of HPTPbeta activity as a "cause" of the angiogenesis mediated disorder or biological manifestation, whether the HPTPbeta is modulated genetically, by infection, by autoimmunity, trauma, biomechanical causes, lifestyle, or by some other causes; (2) The modulated HPTPbeta activity is part of the observable manifestation of the disease or disorder. That is, the disease or disorder is measurable in terms of the modulated HPTPbeta activity. From a clinical standpoint, modulated HPTPbeta activity indicates the disease, however, HPTPbeta activity need not be the "hallmark" of the disease or disorder; (3) The modulated HPTPbeta activity is part of the biochemical or cellular cascade that results in the disease or disorder. In this respect, inhibiting or stimulating of HPTPbeta (per the respective therapeutic goal) interrupts the cascade, and thus controls the disease; (4) The angiogenesis mediated disease or disorder is not the result of modulation in HPTPbeta activity, but modulation of the HPTPbeta activity would result in amelioration of the disease. "Modulation in HPTPbeta activity," as used herein, encompasses both unwanted or elevated HPTPbeta activity and desired or reduced HPTPbeta activity. As used herein, "angiogenesis mediated disorders" include: (1) those disorders, diseases and/or unwanted conditions which are characterized by unwanted or elevated angiogenesis, or (2) those disorders, diseases and/or unwanted conditions which are characterized by wanted or reduced angiogenesis.

Treatment of angiogenesis mediated disorders

Treatment of disorders mediated by elevated angiogenesis

The agents screened by the present invention may be used in a method for the treatment of a disorder mediated by elevated angiogenesis. The agents identified by the present invention may be used to treat diseases like diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's disease, vein or artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus

erythematosis, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma, post-laser complications, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy. Agents screened by of the present invention can also treat diseases associated with chronic inflammation such as Crohn's disease and ulcerative colitis, psoriasis, sarcoidosis and rheumatoid arthritis. Other diseases that can be treated according to the present invention are hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

Treatment of disorders mediated by reduced angiogenesis

In one aspect, an agent may be used in a method for the treatment of a disorder mediated by reduced angiogenesis. As expected, this involves stimulated angiogenesis to treat a disease, disorder, or condition. It is likely that an agent that inhibits HPTPbeta would be used for treatment of an angiogenesis mediated disorder. The disorder is one characterized by tissue that is suffering from or is at risk of suffering from ischemic damage, infection, and/or poor healing, which results when the tissue is deprived of an adequate supply of oxygenated blood due to inadequate circulation (ischemic tissue).

***In silico* Screening of Compounds**

In the present invention, it is possible to carry out virtual screening for drugs using the above-described atomic coordinates or coordinates derived therefrom.

Briefly, the atomic coordinates of the three-dimensional structure elucidated by the invention are input into a computer so that images of the structure and various parameters are shown on the display. Then, the resultant data are input into a virtual compound library. Since a virtual compound library is contained in a virtual screening software such as DOCK-4 (Kuntz, UCSF), the above-described data may be input into such a software. Candidate drugs may be searched for, using a three-dimensional structure database of virtual or non-virtual drug candidate compounds, such as MDDR (Prous Science, Spain).

The potential stimulating/inhibitory or binding effect (i.e., interaction or association) of a compound may be analyzed prior to its actual synthesis and testing by the use of computer modeling

techniques. If the theoretical structure of the given compound suggests insufficient interaction and association between it and HPTPbeta, synthesis and testing of the compound may be obviated. However, if computer modeling indicates a strong interaction, the molecule may then be synthesized and tested for its ability to bind to or stimulate/inhibit HPTPbeta using various methods known in the art or as described in the co-pending application. In this manner, synthesis of inoperative compounds may be avoided.

Agonist/antagonist or binding drug candidates of HPTPbeta may be computationally evaluated and designed by means of a series of steps in which chemical entities or fragments are screened and selected for their ability to bind with individual binding sites or combinations thereof (e.g.,P0, P+1, and/or P-1) or other areas of HPTPbeta.

One skilled in the art may use any of several methods to screen chemical entities or fragments for their ability to associate with HPTPbeta and more particularly with the specific binding sites. This process may begin by visual inspection of, for example, the active site on the computer screen based on the HPTPbeta coordinates in any of the Figures 7-304. Selected fragments or chemical entities may then be positioned in a variety of orientations, or docked, within an individual binding site of HPTPbeta as defined supra. Docking may be accomplished using software such as QUANTA, SYBYL, followed by energy minimization and molecular dynamics with standard molecular mechanics forcefields, such as CHARMM and AMBER.

Specialized computer programs may also assist in the process of selecting fragments or chemical entities. These include: (1) GRID (Goodford, P. J., "A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules" J. Med. Chem., 28, pp. 849-857 (1985)), available from Oxford University, Oxford, UK; (2) MCSS (Miranker, A. and M. Karplus, "Functionality Maps of Binding Sites: A Multiple Copy Simultaneous Search Method" Proteins: Structure, Function and Genetics, 11, pp. 29-34 (1991)), available from Molecular Simulations, Burlington, Mass; (3) AUTODOCK (Goodsell, D. S. and A. J. Olsen, "Automated Docking of Substrates to Proteins by Simulated Annealing" Proteins: Structure. Function, and Genetics, 8, pp. 195-202 (1990)), available from Scripps Research Institute, La Jolla, Calif.; and (4) DOCK (Kuntz, I. D. et al., "A Geometric Approach to Macromolecule-Ligand Interactions" J. Mol. Biol., 161, pp. 269-288 (1982)), available from University of California, San Francisco, Calif; (5) GLIDE available from Schrodinger Inc.; (6) FlexX available from Tripos Inc;

(7) GOLD (Jones et al., J. Mol. Biol., 245, 43-53, 1995), available from the Cambridge Crystallographic Data Centre.

Once suitable chemical entities or fragments have been selected, they can be assembled *in silico* or synthesized into a single drug candidate. *In silico* assembly may proceed by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure coordinates of HPTPbeta. This would be followed by manual model building using software such as QUANTA or SYBYL. Chemical syntheses are by those methods well-known in the art.

Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include the following: (1) CAVEAT (Bartlett, P. A. et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules". In Molecular Recognition in Chemical and Biological Problems", Special Pub., Royal Chem. Soc., 78, pp. 182-196 (1989)), available from the University of California, Berkeley, Calif.; (2) 3D Database systems such as MACCS-3D (MDL Information Systems, San Leandro, Calif.). This area is reviewed in Martin, Y. C., "3D Database Searching in Drug Design", J. Med. Chem., 35, pp. 2145-2154 (1992)); and (3) HOOK (available from Molecular Simulations, Burlington, Mass.).

Instead of proceeding to build an HPTPbeta agonist or antagonist in a step-wise fashion one fragment or chemical entity at a time as described above, drug candidates useful for the treatment of angiogenesis mediated disorders may be designed as a whole or "*de novo*" using either an empty active site or optionally including some portion(s) of a known agonist or antagonist. These methods include the following: (1) LUDI (Bohm, H.-J., "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", J. ComR. Aid. Molec. Design, 6, pp. 61-78 (1992)), available from Biosym Technologies, San Diego, Calif.; (2) LEGEND (Nishibata, Y. and A. Itai, Tetrahedron, 47, p. 8985 (1991)), available from Molecular Simulations, Burlington, Mass.; (3) LeapFrog (available from Tripos Associates, St. Louis, Mo.). Examples of known agonist or antagonists are described in WO 02/26774 A2.

Once a compound has been designed or selected by the above methods, the efficiency with which that compound may bind to HPTPbeta may be tested and optimized by computational evaluation. For example, an effective HPTPbeta agonist must preferably demonstrate a relatively small difference in energy between its bound and free states (i.e., a small deformation energy of binding). Thus, the most efficient HPTPbeta agonist should preferably be designed with deformation

energy of binding of not greater than about 10 kcal/mole, preferably, not greater than 7 kcal/mole. HPTPbeta agonists may interact with the enzyme in more than one conformation that is similar in overall binding energy. In those cases, the deformation energy of binding is taken to be the difference between the energy of the free compound and the average energy of the conformations observed when the agonist binds to the enzyme.

A compound designed or selected, as binding to HPTPbeta may be further computationally optimized so that in its bound state it would preferably lack repulsive electrostatic interaction with the target enzyme. Such non-complementary (e.g., electrostatic) interactions include repulsive charge-charge, dipole-dipole, and charge-dipole interactions. Specifically, the sum of all electrostatic interactions between the compound and the enzyme when the compound is bound to HPTPbeta, preferably make a neutral or favorable contribution to the enthalpy of binding.

Specific computer softwares are available in the art to evaluate compound deformation energy and electrostatic interaction. Examples of programs designed for such uses include: Gaussian 92, revision C [M. J. Frisch, Gaussian, Inc., Pittsburgh, Pa. ©1992]; AMBER, version 4.0 [P. A. Kollman, University of California at San Francisco, ©1994]; QUANTA/CHARMM [Molecular Simulations, Inc., Burlington, Mass. ©1994]; and Insight II/Discover (Biosysm Technologies Inc., San Diego, Calif. ©1994). Other software packages will be known to those skilled in the art.

Once an HPTPbeta -binding compound has been optimally selected or designed, as described above, substitutions may then be made in some of its atoms or side groups in order to improve or modify its binding properties. Generally, initial substitutions are conservative, i.e., the replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group. It should, of course, be understood that components known in the art to alter conformation should be avoided. Such substituted chemical compounds may then be analyzed for efficiency of fit to HPTPbeta by the same computer methods described in detail, above.

Crystallographic Evaluation Of Chemical Entities For Binding To HPTPbeta

For the first time, this invention allows one skilled in the art to study the binding of chemical entities to HPTPbeta by exposing either individual compounds or mixtures of compounds (such as may be obtained from combinatorial libraries) into HPTPbeta crystals or, alternatively, by co-crystallization of the substances of interest with HPTPbeta, using methods known to those of ordinary skill in the art, and the crystallization conditions based on those described in the following examples. Acquisition and analysis of X-ray diffraction data from these crystals can be then

performed using standard methods. If substance or substances bind to HTPTbeta then positive difference electron density will be observed in the Fourier maps calculated using the X-ray diffraction intensities and phases obtained from the HPTPbeta models presented in Figures 7-304. Models of the chemical entities can than be built into the electron density using standard methods and the resulting structures can be refined against the X-ray diffraction data, providing experimental data describing the interaction of the molecules of interest with the enzyme. Those skilled in the art can use these models to design HPTPbeta inhibitors based either on purely structural data or on combination of structural data with enzyme-activity based structure-activity relationship and *in silico* drug design.

EXAMPLES

1. Cloning and expression of the catalytic domain of HPTPbeta

The intracellular domain (ICD) of HPTPbeta (SEQ ID NO: 3) is cloned from human fetal heart cDNA containing the full gene of HPTPbeta (SEQ ID NO: 1) by PCR using Advantage Polymerase (Clontech) and the primers:

Beta-FOR2:	5'-GATCGACCATTATCTGTCCAC-3'	SEQ ID NO: 8
Beta-REV2:	5'-CAGGAGCTCTTCAGGTACAT-3'	SEQ ID NO: 9

under the following reaction conditions: 1 cycle at 95°C for 1 minute, 30 cycles at 94°C for 30 seconds and 62°C for 2 minutes, and a final cycle of 62°C for 3 minutes. PCR products are subcloned into pPCRScript vector (Stratagene) and sequenced, revealing 2 silent mutations in the HPTPbeta ICD clone, one at base pair (bp) 5372 (C to T, a Glycine residue) and the other at bp 5895 (C to T, a Tyrosine residue) (nucleotides are numbered from bp #1 in SEQ ID #1, which means that the initiator methionine in SEQ ID #2 corresponds to the codon beginning at base pair #31 in SEQ ID NO 1).

The sequence [SEQ ID NO: 5] encoding base pairs 5014 to 5949 of SEQ ID NO: 1 is cloned into the vector pMALc2x (New England Biolabs) using the following oligonucleotides:

5' primer:	5'-CGAGCATACGTAGATCGACCATTATCTGTCC-3'	SEQ ID NO: 10
3' primer:	5'-CGAGCAAGCTTATTATTGTTCACTCCGTAGC-3'	SEQ ID NO: 11

The HPTPbeta truncated gene [SEQ ID NO: 5] (Wang, Y. & Pallen, C.J. The journal of Biological Chemistry, 267(23), pp16696-16702, 1992) is amplified with these primers by PCR using the pPCRScript plasmid described above as the template, digested with SnaB1 and HindIII and ligated to pMAL-c2x that is pre-digested with Asp700 and HindIII to create plasmid pMAL-c2x-PTPbeta(5014-5949). The protein construct thus encoded is maltose-binding protein from *Escherichia coli* (MBP) followed by a Factor Xa cleavage site followed by base pairs 5014 to 5949 of HPTPbeta SEQ ID NO: 1. A six-histidine tag is added to the carboxy terminus using the QuikChange Site Directed Mutagenesis kit (Stratagene) and the following primers:

5'-GAAAGCTACGGAGTGAACAACATCATCATCATCATTAATAAGCTTGGCACTGG-3' SEQ ID NO: 12

5'-CCAGTGCCAAGCTTATTAATGATGATGATGATGTTGTTCACTCCGTAGCTTTC-3'. SEQ ID NO: 13

The coding sequence of the clone (pMAL-c2x-PTPbeta(5014-5949)His6) is verified by DNA sequence analysis. Finally, the Factor Xa site is changed to a TEV protease cleavage site using the QuikChange Site Directed Mutagenesis kit and the following primers:

5'-AACAACAACCTCGGGGAGAATCTTTATTTTCAGGGCGATCGACCATTATCTG-3' SEQ ID NO: 14

5'-CAGATAATGGTCGATCGCCCTGAAAATAAAGATTCTCCCCGAGGTTGTTGTT-3'. SEQ ID NO: 15

The protein construct thus encoded is maltose-binding protein from *Escherichia coli* (MBP) followed by a TEV protease cleavage site, followed by base pairs 5014 to 5949 of HPTPbeta SEQ ID NO: 1, followed by a six-histidine tag. After TEV protease cleavage, the resulting protein contains a non-native Glycine residue on the amino-terminus and a six-histidine tag on the carboxy-terminus (SEQ ID 7).

The coding sequence of the final clone (pMAL-TEV-PTPbeta(5014-5949)His6) is verified by DNA sequence analysis and used for recombinant protein production. *Escherichia coli* BL21-RIL cells (Stratagene) are used as the host strain. Bacteria are grown in a 10-liter fermenter, using Super Broth medium (30g tryptone, 20 g yeast extract, 7.5 g NaCl per liter) supplemented with 0.2%

glucose and 100 mg/L ampicillin at 22C to mid-log phase, at which point the bacteria are induced with 0.5 mM β-isopropyl thiogalactopyranoside and harvested by centrifugation 16 hours after induction.

2. Purification of the catalytic domain of HPTPbeta

26 grams of cell pellet containing the overexpressed protein are suspended in 287.5 ml of 50mM NaH_2PO_4, 300mM NaCl, 10mM imidazole, 5mM β-mercaptoethanol, , pH 8.0 (Buffer A) containing 4 "Complete-EDTA free" protease inhibitor tablets (Roche). Cells are lysed during 2 passes through a French press at 12000 psi, at 4°C. The lysate is centrifuged for 40 minutes at 17000rpm, using a JA-17 (Sorvall) rotor at 4°C. Resulting supernatant (295ml) is loaded at 10ml/min, 4°C on a 73 ml Ni-NTA column which is pre-equilibrated with Buffer A. The column is washed with 200ml of 75mM Imidazole in Buffer A and the protein is eluted with 175mM of imidazole in Buffer A (6ml fractions, 10ml/min). Fractions containing the fusion protein are pooled based on Coomassie Blue-stained SDS-PAGE analysis. The pool is diluted to 515ml with pure water to final conductivity of 10.42mS/cm (approximately 125-135mM NaCl).

Diluted fusion protein is loaded onto a 150ml Resource Q15 (Pharmacia) column pre-equilibrated with 10mM Tris·HCl, 2mM DTT, pH 7.2. The protein is eluted with a 22.5-29% linear gradient of 10mM Tris·HCl, 2mM DTT, 1M NaCl, pH 7.2 (10 ml fractions are collected at 10ml/min flow rate). Fractions are pooled based on SDS-PAGE analysis.

N-Octyl β-glucopyranoside (NOG) and dithiothreitol (DTT) are added to the pool resulting in final concentrations of 0.25% and 2mM, respectively. Approximately 1.5 mg of recombinant Tobacco Etch Virus protease (TEV protease, Invitrogen) are added to the pool (using ~1/200 weight ratio of protease to substrate) and the reaction mixture is incubated with stirring for 17 hrs at 4°C. SDS-PAGE analysis reveals ~65% cleavage efficiency. Further addition of TEV protease does not result in additional cleavage.

Reaction mixture is adjusted to the final conductivity of 10.4 mS/cm by addition of 10mM Tris·HCl, 2mM DTT, 0.25% NOG, pH 7.2 (Buffer B) and loaded on a 150ml Resource Q15 column pre-equilibrated with Buffer B. Proteins are eluted with a 19-24% linear gradient of 1M NaCl in Buffer B. Unfortunately, the fusion protein and the cleaved PTPβ catalytic domain [SEQ ID NO: 7] do not separate well using this method. Fractions containing the cleaved catalytic domain are pooled based on SDS-PAGE analysis.

The pool containing the PTPbeta catalytic domain [SEQ ID NO: 7] as well as some of the uncleaved fusion protein is concentrated down to 4.5ml using a YM-10 membrane in an Amicon stirred cell and slowly passed through a 3ml amylose resin (Quiagen) column pre-equilibrated with Buffer B. Flow-through is collected and the column is washed with 3ml of Buffer B. Combined flow-through and wash are loaded on a 48X5.0cm Superdex 75 prep grade column, pre-equilibrated with Buffer B. The column is eluted with Buffer B at 2 ml/min while 10 ml fractions are collected. Two peaks are observed indicating good separation between the full-length fusion protein and the HPTPbeta catalytic domain. Fractions are analyzed by SDS-PAGE and those found to contain pure protein are pooled. HPTPbeta catalytic domain [SEQ ID NO: 7] is concentrated to 9.8 mg/ml using an Amicon stirred cell equipped with YM-10 membrane. Total yield of HPTPbeta catalytic domain is 58.6 mg based on the OD_{280} measured in 6M guanidine-hydrochloride, pH 8.0.

3. Crystallization of the catalytic domain of HPTPbeta, collection of X-ray diffraction data, and structure solution

HPTPbeta catalytic domain [SEQ ID NO: 7] is crystallized in hanging drops via the sparse-matrix approach using crystallization screens manufactured by Hampton Research and Emerald Biostructures (currently DeCode Genetics). Several polyethylene glycol (PEG)/magnesium chloride conditions are identified as crystallization leads, which are eventually refined to the following condition: 18% PEG8000, 100 mM TRIS-HCl pH 7.5, 1% β-mercaptoethanol (BME), 0.2 M MgCl2 in 6µl drops containing equal amounts of protein and reservoir solutions. Under these conditions, crystals usually appear after 2-5 days, reach their maximum size of 0.2x0.2x0.3 mm in 6-8 days and decay within 3-4 weeks after set-up. The best crystals are grown by streak-seeding, which results in more reliable nucleation. For structural studies, individual protein crystals are cryoprotected by immersion into Paratone-N oil followed by flash cooling in a stream of nitrogen gas at 100K.

Two clearly different crystal morphologies are observed – one is subsequently identified as orthorhombic and the other as monoclinic crystal form. Laboratory X-ray source equipped with a CCD X-ray detector is used to collect 2.3 Å data from the orthorhombic crystals, which are found to belong to the space group $P2_12_12_1$ with unit cell dimensions of a=39.25 Å, b=71.13 Å, and c=119.91 Å, α=90°, β=90°, γ=90°, and one molecule in the asymmetric unit (a.u.). Programs of the HKL2000 suite are employed to index, integrate, and scale the diffraction data.

The crystal structure is solved via molecular replacement (CCP4 program AMoRe) (Collaborative Computational Project, Number 4. 199 Acta Cryst. D50, 760-763; and Navaza,J. *Acta Cryst.* A50,

157-163 (1994) using the published structure of PTPμ from the Protein Data Bank (PDB), PDB code 1RPM as the search model. After multiple rounds of manual rebuilding (program O) and refinement (programs Refmac and SHELXL) the crystal structure of the enzyme contains residues 24 to 305, as well as 115 water molecules, and had the R_{work} of 21.0%, and R_{free} of 26.2%. Concomitantly we are able to collect high-resolution data for both the orthorhombic and the monoclinic crystal forms of the enzyme using a synchrotron radiation source (beam lines 17-ID and 17-BM of the Advanced Photon Source at the Argonne National Laboratory – APS/ANL). The monoclinic form of the enzyme is found to belong to the space group $P2_1$ with unit cell dimensions of a=61.89 Å, b=71.53 Å, and c=70.35 Å, α=90°, β=93.25°, γ=90°. Using synchrotron data we are able to refine the structure of the enzyme in the orthorhombic crystal form to 1.75 Å resolution (R_{work}=19.0%, R_{free}=22.0%) and the monoclinic crystal form (which has two protein molecules in the a.u.) to 1.9 Å resolution (R_{work}=20.0%, R_{free}=24.0%). Validation of structures using programs PROCHECK and SFCHECK does not reveal any geometric abnormalities. Finished structures contain residues 19 to 310 as numerous water molecules.

Proprietary X-ray diffraction data were collected at beamlines 17-ID and 17-BM in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source. These facilities are supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Illinois Institute of Technology (IIT), executed through IIT's Center for Synchrotron Radiation Research and Instrumentation. Use of the Advanced Photon Source was supported by the U. S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

4. Exposing inhibitors into HPTPbeta catalytic domain crystals, and structure solution of the resulting complexes

To study the interaction of ligands with PTPβ we expose (soak) various molecules of interest into both the orthorhombic and the monoclinic crystals at a concentration of 1-10 mM in the crystallization buffer, using exposure times from 2 to 24 hours. Monoclinic crystals of the enzyme can be readily soaked (2-4 hours) with compounds of interest – and the resulting soaked crystals usually (with a notable exception that is outlined later) do not deteriorate. True ligands and/or inhibitors can be unambiguously identified, by analyzing electron density maps calculated using X-ray diffraction data collected from the soaked crystals. Interestingly, when molecules that belong to

the family of Compound 2 are soaked into the monoclinic crystals, the latter deteriorate to the point where collection of X-ray diffraction data is impossible. Fortunately, the orthorhombic crystals of PTPbeta catalytic domain can be successfully soaked with compounds of this class, which is particularly interesting in view of the fact that the orthorhombic crystals are nigh impossible to soak with many other classes of compounds – even 24-hr soaks do not result in small molecule penetration into the crystal lattice.

X-ray diffraction data from soaked crystals are collected and processed at APS/ANL in the same manner as described above. X-ray crystal structures of the complexes are solved via molecular replacement (AMoRe) using corresponding native structures for each crystal form as search models. Several rounds of manual rebuilding (O) and refinement (Refmac, SHELXL) are employed, after which the inhibitor molecules are built into the electron density (Quanta, SPARTAN) and refined. The geometry of the structures is analyzed using programs PROCHECK and SFCHECK.

5. HPTPbeta catalytic domain in two space groups – overview of the unliganded structure

The two final models of the protein contain residues 19-310, which are clearly defined in the electron density maps of the orthorhombic and monoclinic forms, respectively. Loop 89-96 is entirely disordered in the orthorhombic form (and therefore is omitted from the final model) and is mostly disordered in the monoclinic form. The orthorhombic crystal form of the PTPbeta catalytic domain contains one protomer in a.u. whereas the monoclinic form contains two protomers in the a.u.. Root mean square deviation (rmsd) of the protein backbone atoms of the two molecules found in the monoclinic a.u. is 0.45 Å, whereas rmsd of the orthorhombic PTPbeta catalytic domain and the monoclinic protomers is 0.55-0.66 A. Upon close inspection, it is evident that there are no major structural differences between the three molecules – the changes are confined to minor movements of the loops and rearrangements of conformationally unrestrained side chains of surface-exposed amino-acids. Therefore it is sufficient to supply the overall description of the unliganded PTPbeta catalytic domain performed using the best-defined (highest resolution) protomer - the one found in the orthorhombic crystal form.

PTPbeta belongs to a broad family of PTPases that are extremely dissimilar in terms of their biological function, intracellular localization, and domain structure. These very diverse enzymes have one feature in common, namely their catalytic domain, the fold of which is preserved throughout this whole class of PTPases. Historically, the first human PTPase to be discovered and

studied is PTP-1B, which is a soluble single-domain phosphatase that was first identified in human placenta in 1989. Because of its early discovery and biological significance, PTP-1B is the most studied PTPase to-date, with over 40 X-ray crystal structures, both unliganded and liganded, available in the PDB. PTP-1B structure is therefore considered to be an archetype of the catalytic domain of this class of PTPases. Here, we use comparisons between PTPbeta catalytic domain and PTP-1B (PDB code 2HNP) structures to illustrate the relationship between our crystal structure of the PTPbeta catalytic domain and the rest of the PTPases of this class.

Similar to PTP-1B, the crystal structure of PTPbeta catalytic domain reveals a common PTPase fold, consisting of two closely-packed compartments: the alpha-helical domain and the beta-sheet one (Figure 1). When the backbone atoms of the two enzymes are superimposed, the two structures fit with an rmsd of 1.3 A for the matching atoms. In this case, however, the rmsd does not adequately represent the dissimilarity of the two structures. The macroscopic and microscopic differences between PTP-1B and PTPbeta catalytic domain are closely examined below. On the level of backbone, the differences between the two structures are:

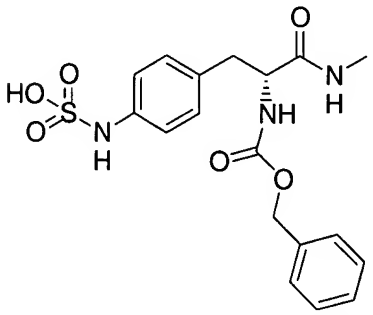
- The N-terminus – PTPbeta catalytic domain structure has 12 more ordered residues (residues 19-31) than PTP-1B structure, and the first two alpha-helices (residues 32-55) of the two proteins occupy different positions.
- The 106-111 loop of PTPbeta catalytic domain is placed differently than the corresponding 74-80 loop of PTP-1B.
- The first beta-strand and its beta-turn (residues 161-168 of PTPbeta catalytic domain) are shifted with respect to their PTP-1B analogues (130-139)
- The geometry of the 191-197 loop of PTPbeta catalytic domain is very different from its PTP-1B analogue 162-167.
- Loop 233-238 adopts a radically different conformation from its PTP-1B equivalent 202-209.
- Residues 262-277 and the C-terminal a-helix 291-310 are shifted with respect to the position of their equivalents (residue 252-270 and a-helix 264-282) in PTP-1B.

The active site of the enzyme (residues 152-153, 244-253, 288-290, and 293) is located at the junction of the two domains and is occupied by four water molecules. A characteristic loop, containing a Trp-Pro-Asp triad (the WPD loop, residues 208-214) is located near the binding site. In PTP-1B and other phosphatases this loop is known to adopt a different conformation upon binding of

ligands in the active site. As will be shown in the next example this conformation change also takes place when PTPbeta catalytic domain binds to ligands (Figure 2). The WPD loop of PTPbeta catalytic domain contains a His212 instead of Phe181 in PTP-1B, and the orientation of PTPbeta catalytic domain Trp209 is different from that of its PTP-1B analogue Trp179.

Additional small, but important distinctions between PTPbeta catalytic domain and PTP-1B are further discussed in the example 7.

6. Synthesis of Compounds 1 ((S)-[1-Methylcarbamoyl-2-(4-sulfoamino-phenyl)-ethyl]-carbamic acid benzyl ester) and Compound 2 ({2-(4-Hydroxy-phenyl)-1-[1-methylcarbamoyl-2-(4-sulfoamino-phenyl)-ethylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester)



(S)-[1-Methylcarbamoyl-2-(4-sulfoamino-phenyl)-ethyl]-carbamic acid benzyl ester (Compound 1)

Boc-D-Phe(4-NO_2)-NMe: Boc-D-Phe(4-NO_2)-OH (4.0 g, 12.9 mmol) is dissolved in anhydrous tetrahydrofuran (20 mL) with 4-methylmorpholine (1.56 mL, 14.2 mmol). Isobutylchloroformate (1.84 mL, 14.2 mmol) is dropwise added at 0°C and the mixture is stirred for 1 hr. at 0°C. Methylamine (12.9 mL, 2.0 M in tetrahydrofuran) is added dropwise at 0°C and the mixture is stirred for 18 hr. at room temperature. The mixture is then recrystallized from 1:1 DCM:methanol to give a white solid.

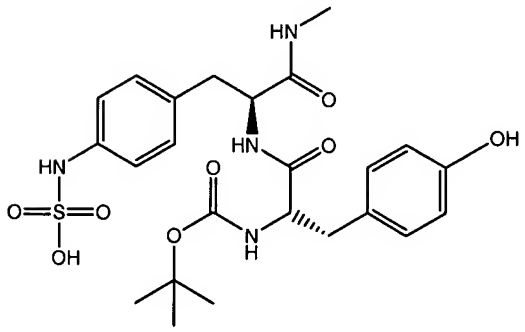
H-D-Phe(4-NO_2)-NMe: Boc-D-Phe(4-NO_2)-NMe (1.5g, 4.64 mmol) is dissolved in HCl (10 mL, 4.0 M in 1,4-dioxane), and the resulting mixture is stirred at room temperature for 1 hr. Ether (60 mL) is added to the mixture and the resulting precipitate is collected by filtration to yield pure white product.

CBZ-D-Phe(4-NO_2)-NMe: H-D-Phe(4-NO_2)-NMe (410 mg, 1.84 mmol) is dissolved in anhydrous DCM (10 mL) and diisopropylethylamine (0.352 mL, 2.02 mmol). Benzyl chloroformate

(0.263 mL, 1.84 mmol) is added dropwise at 0°C, the mixture is allowed to warm to room temperature and is stirred for 72 hr. The solution is partitioned between DCM and 1N HCl. The organic layer is washed with brine, dried over $MgSO_4$, filtered and evaporated to give crude white solid.

CBZ-D-Phe(4-NH_2)-NMe: CBZ-D-Phe(4-NO_2)-NMe (80 mg, 0.224 mmol) is dissolved in EtOAc: ethanol (1:1, 2 mL) and tin(II) chloride dihydrate (252mg, 1.12 mmol) is added. The mixture is stirred at room temperature for 18h. The reaction is partitioned between EtOAc (25 mL) and 1N NaOH (25 mL). The organic layer is washed twice more with 1N NaOH (25 mL). The combined organics were dried over $MgSO_4$, filtered and evaporated to give pure yellow oil.

(S)-[1-Methylcarbamoyl-2-(4-sulfoamino-phenyl)-ethyl]-carbamic acid benzyl ester: In a dry flask 0.107g of the aniline compound is dissolved in 2 mL pyridine. To this solution is added 0.156g of sulfurtrioxide-pyridine complex. The mixture is stirred 5 minutes then diluted with 25 mL of 7% ammonium hydroxide. The mixture is evaporated down to an off-white solid and purified to provide 0.056g of product as its ammonium salt. $^1H(D_2O)$: δ7.26-7.20 (m, 3H), 7.11-6.96 (m, 6H), 4.90-4.78 (m, 2H), 4.08 (t, 1H, *J*=8.3 Hz), 2.84-2.66 (m, 2H) 2.50 (s, 3H)



{2-(4-Hydroxy-phenyl)-1-[1-methylcarbamoyl-2-(4-sulfoamino-phenyl)-ethylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester (Compound 2)

Boc-Phe(4-NO_2)-NMe: Boc-Phe(4-NO_2)-OH (10.0 g, 32.3 mmol) is dissolved in anhydrous tetrahydrofuran (32.2 mL) with 4-methylmorpholine (3.90 mL, 35.4 mmol). Isobutylchloroformate (4.18 mL, 32.3 mmol) is dropwise added at 0°C and the mixture is stirred for 1 hr. at 0°C. Methylamine (332.3 mL, 2.0 M in tetrahydrofuran) is added dropwise at 0°C and the mixture is stirred for 18 hr. at room temperature. The mixture is then recrystallized from 1:1 DCM:methanol to give 6.69 g pure white solid.

H-Phe(4-NO_2)-NMe: Boc-D-Phe(4-NO_2)-NMe (1.5g, 4.64 mmol) is dissolved in HCl (10 mL, 4.0 M in 1,4-dioxane), and the resulting mixture is stirred at room temperature for 1 hr. Ether (60 mL) is added to the mixture and the resulting precipitate is collected by filtration to yield pure white product.

{2-(4-Hydroxy-phenyl)-1-[1-methylcarbamoyl-2-(4-nitro-phenyl)-ethylcarbamoyl]-ethyl} carbamic acid tert-butyl ester: H-L-Phe(4-NO_2)-NMe (200 mg, .770 mmol) is dissolved in 1 mL DMF. Diisopropylethylamine (209 mg, 1.62 mmol), EDC (162 mg, .847 mmol), HOBt.H_2O (130 mg, .847 mmol), and Boc-Tyr (238 mg, .847 mmol) are added and the mixture is stirred for 18 hr. at 20°C. The mixture is partitioned between water and EtOAc (2 x 60 mL). The organics are combined and washed with brine, dried over $MgSO_4$, filtered and evaporated to give crude product. Purification by flash chromatography, which is eluted with 97:3 DCM:methanol to give pure white solid.

{2-(4-Hydroxy-phenyl)-1-[1-methylcarbamoyl-2-(4-amino-phenyl)-ethylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester: {2-(4-Hydroxy-phenyl)-1-[1-methylcarbamoyl-2-(4-nitro-phenyl)-ethylcarbamoyl]-ethyl} carbamic acid tert-butyl ester (300, .617 mmol) is dissolved in methanol (10 mL). To this was added palladium on carbon (10% by weight, 100 mg). The reaction is placed under a hydrogen atmosphere until reaction is complete (tlc). The catalyst is removed by filtration and the filtrate is concentrated to provide the amine, which is used without purification.

{2-(4-Hydroxy-phenyl)-1-[1-methylcarbamoyl-2-(4-sulfoamino-phenyl)-ethylcarbamoyl]-ethyl}-carbamic acid tert-butyl ester: In a dry flask the aniline compound is dissolved in 2 mL pyridine. To this solution is added 0.295g of sulfurtrioxide-pyridine complex. The mixture is stirred 5 minutes then diluted with 50 mL of 7% ammonium hydroxide. The mixture is evaporated down to an off-white solid and purified to provide 0.062g of product as its ammonium salt. $^1H(D_2O)$: δ7.00-6.87 (m, 6H), 6.69 and 6.66 (d, 2H, J=9.3 Hz), 4.28 (t, 1H, J=8.0), 4.05 (t, 1H, J=8.7 Hz), 2.81-2.65 (m, 4H), 2.47 (s, 3H), 1.21 (s, 9H)

7. HPTPbeta catalytic domain in complex with ligands

Crystal structures are a very useful tool for design of PTPbeta inhibitors. As an illustration, we describe here two different modes of binding of PTPbeta inhibitors, determined through solving high-resolution crystal structures of PTPbeta catalytic domain complexes with the representative members of these classes – Compound 1 and Compound 2.

Additional similarities and differences between the structure of the ligand-binding site of PTP-1B and PTPbeta catalytic domain become apparent upon comparison of the ligand-bound structures of PTPbeta catalytic domain with the ligand-bound structure of PTP-1B (PDB code 2HNP).

On the one hand, as can be seen from Figure 3, the two sulfamic acid-based inhibitors described here bind with the phenyl sulfamic acid portion of the molecule occupying the P(0) pocket (the active site) of the enzyme. The sulfamic acid moiety is connected to the active site by an extensive network of hydrogen bonds (Figure 4a), closely resembling the binding of phosphotyrosine phosphate group to previously studied PTPases, as exemplified in Figure 5 showing superposition of phosphotyrosine bound in the active site of PTP-1B (PDB code 1PTV) with the sulfamic acid bound in the active site of PTPbeta catalytic domain. The binding of sulfamic acids in P(0) pocket is accompanied by the closure of the WPD loop (Figure 2) which brings Asp211 within hydrogen-bonding distance to sulfamic acid nitrogen and results in sandwiching of the phenyl between residues His212 and Ala247 – again, resembling binding of phosphotyrosine to the active site of PTPases (e.g. PTP-1B). The phenyl group of the ligand participates Van der Waal's interactions with residues His286, His212, Ala247, Val249, and Gln289 (Figure 4b). It is thus easy to assume that the phenyl sulfamic acid mimics the phosphotyrosine side chain that is the natural target of PTPbeta.

On the other hand, there are significant differences in the structure of the binding sites between PTPbeta catalytic domain and PTP-1B. In particular these include:

- Orientation of Gln289 is different from that of its PTP-1B analogue Gln262
- Orientation of the side chain of His212 is different from that of its PTP-1B analogue Phe182
- PTPbeta catalytic domain has Asn76-Asp75 instead of Asp48-Arg47 in PTP-1B, which results in different orientation of both amino acid side chains, as well as slight but significant difference in the geometry of the backbone in that region of the protein
- In PTPbeta catalytic domain, His286 occupies the mouth of the P(+1) pocket, instead of Gly259 in PTP-1B. This bulky amino acid is likely to modulate access to the P(+1) pocket of the enzyme
- Differences in the 147-155 stretch conformation as compared to its PTP-1B analogue are likely to result in different specificity of the P(-1) pocket

- Conformation of the 48-58 region of PTPbeta catalytic domain is different from that of its equivalent in PTP-1B, likely resulting in altered specificity of the P(-1) pocket of the enzyme

In both PTPbeta catalytic domain complex structures presented here, the sulfamic acid moiety and the phenyl ring bind to the enzyme in a very similar manner, however the binding mode of the remainder of these two inhibitors to the protein is quite different, as follows:

P(0) pocket: Whereas Compound 1 interacts with His212 through the C-terminal amide carbonyl and through the phenyl ring, in the structure of the Compound 2 complex, His212 side chain is abstracted away from the binding site as a result of an interaction with the tyrosine side chain of the inhibitor (Figure 6). The N-terminal amide carbonyl of Compound 2 forms a hydrogen bond with the nitrogen of the Asn76 side chain whereas there is no direct interaction between Asn76 and Compound 1.

P(-1) pocket: The N-terminal carbobenzoxy group of Compound 1 participates in extensive Van der Waal's contacts with Arg73 and Tyr74. In contrast, the C-terminal amide of Compound 2 interacts with Tyr74 and to a lesser extent with Arg150.

P(+1) pocket: The only interaction of Compound 1 with this pocket of the enzyme is a superficial VdW contact with Gln289. Compound 2 interacts with the P(+1) pocket through hydrogen bonding of the t-BOC carbonyl with Gln289 side chain, and via extensive VdW interactions of the tert-butyl group with Ile77, Val249, Gln289, Val285, His286, and even Arg56. Notably, His286 is misplaced from its position in the both the unliganded enzyme and in the complex of PTPbeta catalytic domain with Compound 1. In its new position, His286 pi-stacks with the guanidine of Arg281 and forms potential hydrogen bonds with main-chain carbonyls of Val288 and Lys52 or, depending on its protonation state, an ion-pair with Arg56.

In view of the above, it is not surprising that Compound 2, which forms an impressive array of interactions with the enzyme is a more potent inhibitor than Compound 1 which has a much more modest number of interactions with PTPbeta catalytic domain.

Except as otherwise noted, all amounts including quantities, percentages, portions, and proportions, are understood to be modified by the word "about", and amounts are not intended to indicate significant digits.

Except as otherwise noted, the articles "a", "an", and "the" mean "one or more".

All documents cited are, in relevant part, incorporated herein by reference; the citation of any document is not to be construed as an admission that it is prior art with respect to the present invention

While particular embodiments of the present invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope.